The Clark Family Story: Tracing the Cause of Hemophilia

by Trish Strohfeldt

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In collaboration with the Centers for Disease Control and Prevention's
Nicole Dowling and Stacy League

This lesson is for a senior level biology course and emphasizes the relationship between DNA sequences, mutations in DNA and the change in the resulting protein structure and function. Hemophilia A will be used as a real life example of how a mutation in DNA results in a change in a protein's structure and resulting ability to function. In order to complete these activities, the students should have prior knowledge in DNA structure and mutations, protein synthesis, Mendelian genetics and sex-linked traits.

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Summary
This lesson is for a senior level biology course and emphasizes the relationship between DNA sequences, mutations in DNA and the change in the resulting protein structure and function. Hemophilia A will be used as a real life example of how a mutation in DNA results in a change in a protein's structure and resulting ability to function. In order to complete these activities, the students should have prior knowledge in DNA structure and mutations, protein synthesis, Mendelian genetics and sex-linked traits.

Learning Outcomes

- The student will be able to relate a DNA sequences to the resulting protein structure in the factor VIII gene.
- The student will be able to demonstrate the use of restriction enzymes in the identification of a specific DNA sequence and explain how this can be used to diagnosis someone has having the hemophilia factor VIII mutation.
- The student will be able to demonstrate how a protein's structure and function can change with a change in the factor VIII DNA sequence (mutation).
- The student will be able to explain the genetics of hemophilia inheritance and affect on the blood clotting process.
- The student will be able to draw conclusions about the risk of passing on the mutated gene.

Materials

1. Photocopy of introduction and background activity-one per student
2. Photocopied DNA sequence templates-one set per group of 4 students
3. 15 feet of white paper (large roll butcher type) with base pair lengths marked (see diagram-Electro paper gel template)
4. Floralwire-40cm per student
5. Sharpie markers-1 per student
6. Computers with Internet access and RASMOL program
7. Biology textbooks to use as reference materials
8. Lab materials including tape scissors, wire, and markers

Total Duration
4 hours

Procedures

Teacher Preparation
The teacher should prepare photocopies of handouts. Copies will need to be made of the following: Introduction – "Pretest and Clark Family Background"; Step 2 – "Student Template Factor VIII DNA Sequences" (one per group of 4-5), Hemophilia Factor VIII Mutation Activity Student Directions; Step 3 – "Student Directions: Physical Protein Model Building", "Conclusion-Genetic Counselor Report Rubric".

For Steps 2 and 3 organize lab materials (tape, scissors, wire, markers) at lab stations. A computer lab should be reserved for the Step 3 and the RASMOL program should be downloaded and tested. For the activity in Step 2, use large pieces of paper to create the electrophoresis gels with the + and – ends, wells and base pair markers labeled on each. Use the ”Electrophoresis Gel Template Paper” as a guide for drawing this.

This lesson plan should be taught after students have covered the basics of inheritance, including DNA structure, protein structure and synthesis, mutations, Mendelian genetics, and sex-linked traits. For additional information on these topics, visit the Web resources included below.

Web Resources
Title: Online Mendelian Inheritance in Man-Hemophilia A
URL: http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=306700
Description: This Web site is the OMIM hemophilia A entry that includes background information for the teacher on the biochemistry, clinical features and research.

Supplemental Document
Title: Electrophoresis Gel Paper Template
Description: This document is a scaled down drawing of what the teacher will need to create on large white paper for each lab group in order for them to complete the activity. Once the large versions of the gels are created the teacher may re-use these each time the lesson is done.

Introduction
Before beginning the lesson, the “Pretest – Hemophilia Lesson Plan” can be used to assess students' prior knowledge. The “Pretest Answer Key – Hemophilia Lesson Plan” contains answer to the pretest for the teacher’s reference.

Following the pretest, students will be introduced to hemophilia and its inheritance pattern. Using “The Clark Family Background” document, students will read a description of a family and their symptoms and use that information to learn about hemophilia. Students should take notes as the background information is discussed since they will need this information to complete later steps and to include in their conclusion paper. A Web resource is included below to supplement the conversation.

Web Resource
Title: National Hemophilia Foundation
URL: http://www.hemophilia.org/bdi/bdi_types1.htm
Description: This Web site provides background information on hemophilia A (Factor VIII) deficiency. Teachers can use this Web site to supplement their discussion on the Clark family and hemophilia.

Supplemental Documents
Title: Pretest – Hemophilia Lesson Plan
Description: This file is the pretest that can be used to assess the students' knowledge of genetics, hemophilia proteins, and inheritance before the lesson.

Title: Pretest Answer Key – Hemophilia Lesson Plan

Description: This file is the pretest answer key that can be used to discuss the students' responses to the pretest.

Title: The Clark Family Background
Description: This file is the introduction information that gives the students background on the Clark family and the disorder of hemophilia.

Step 2 **Duration: 45 minutes**

Now that the students have been introduced to the Clark family and hemophilia, students will explore the genetic underpinnings of the Clark family's disease. The teacher will organize the students into groups of 3-4 and distribute one "Student Template Factor VIII DNA Sequences" document to each group and the "Factor VIII Mutation Activity Student Directions" worksheet to all students. Following the instructions in the worksheet, the students will cut apart each DNA strand on the template, tape them into the correct order, and label each strand with the source information. The actual DNA sequence for factor XIII can be found on the "NCBI Factor VIII F8 Entry" Web site included below.

Following the student directions, students will be given a restriction enzyme to cut the DNA sequences with. For the teacher's reference, a site that matches nucleotide sequences with restriction enzymes is included below. Answers for the restriction cuts are found in the "Factor VIII DNA Sequences Answer Key" along with other sequence information.

After they have cut the sequence with the restriction enzyme, students will compare the sequences on a simulated gel to determine which of the family members carry the Hemophilia A mutation, which family members have the disease, and which ones are free of the mutation. The gel will be copied onto the large electrophoresis gel drawn by the teacher in the Teacher Preparation Step. "Hemophilia Factor VIII Mutation Activity Answer Key" provides answers to the questions found in the student directions worksheet.

Web Resources

Title: NCBI Factor VIII F8 Entry
URL: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=Nucleotide&dopt=GenBank&val=67083285
Description: This Web site will provide you with actual DNA sequence nucleotide bases for the factor XIII gene.

Title: NEBcutter V2.0 New England Biolabs Inc.
URL: http://tools.neb.com/NEBcutter2/index.php
Description: This Web site allows you to input any DNA nucleotide sequence to find restriction enzyme cut sites along the sequence.

Supplemental Documents

Title: Student Template Factor VIII DNA Sequences
Description: This is the document that contains each family member's DNA strands, which each group will cut out to analyze. Each group will need one copy of this file.

Title: Hemophilia Factor VIII Mutation Activity Student Directions
Description: This document is the student version of the activity and post activity questions that are to be completed as students complete the activity.

Title: Hemophilia Factor VIII Mutation Activity Answer Key

Description: This document is the teacher's answer key to the student activity.

Title: Factor VIII DNA Sequences Answer Key
Description: This document is the teacher answers to the DNA sequences and where the restriction enzyme cuts each DNA strand.

Step 3 **Duration: 1 hours, 30 minutes**

To further their exploration of the genetic underpinnings of the Clark family's hemophilia, the students will use the DNA sequences found in the "Student Template Factor VIII DNA Sequences" document from Step 2 to determine the effect of the mutation in the factor VIII gene on the resulting factor VIII protein structure. Using the instructions "Student Directions: Physical Protein Model Building", students will begin by transcribing and translating their DNA into mRNA, amino acids, and shapes. Then, each student will create a physical model of one of the family member's factor VIII protein and compare the structures. Finally, the students will use a molecular visualization tool called RASMOL to look at the structure of a part of the factor VIII protein. Information about downloading and using RASMOL is found in the Web resources for this step. The student directions worksheet can be evaluated using the "Student Directions: Physical Protein Model Building Answer Key". In addition, the mRNA, amino acid, and shapes can be evaluated using the "Factor VIII DNA Sequences Answer Key" found in Step 2.

Web Resources

Title: RASMOL Quick Start Page
URL: http://www.umass.edu/microbio/rasmol/rasquick.htm
Description: This Web site provides an introduction to using the molecular visualization tool RASMOL. Students can use it before the lesson or during the lesson.

Title: RASMOL Download Page
URL: http://www.umass.edu/microbio/rasmol/getras.htm
Description: This Web site provides information on downloading the free version of RASMOL. Once downloaded, RASMOL can be used to view and modify coordinate files of molecular structures.

Supplemental Document

Title: Student Directions: Physical Protein Model Building
Description: This worksheet directs the students to create the physical model of the mutated and normal version of the factor VIII protein to compare the structure and then infer the effects on the function of the protein in the blood coagulation pathway. Students will transcribe the DNA of each family member into mRNA and then translate into amino acids. The student will then use wire to create a physical model of the protein.

Title: Student Directions: Physical Protein Model Building Answer Key
Description: This document is the answer key to the questions on the student version of the activity.

Conclusion **Duration: 45 minutes**

After students have found the gene responsible for the disorder and have found out which members of the family are affected and which members are carriers in the previous steps the students will then act as genetic counselors. As genetic counselors they will prepare a report for the Clark family that includes the information on living with hemophilia, the affected gene and location, mode of inheritance and risk of future offspring in the family (parents and children) developing hemophilia. To create this report they should use what the have learned in previous

steps and the Web resources included in this step. Students should include Punnett squares and pedigrees in the report. Students will be evaluated using the "Genetic Counselor Report Rubric". This rubric should be given to the students before they begin to create their reports so they know what to include.

Web Resources
Title: Online Mendelian Inheritance in Man-Hemophilia A
URL: http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=306700
Description: This Web site is the Online Mendelian Inheritance in Man (OMIM) hemophilia A entry that includes background information for the student on the biochemistry, clinical features and research for their conclusion paper.

Title: National Hemophilia Foundation
URL: http://www.hemophilia.org/bdi/bdi_types1.htm
Description: This Web site provides background information on hemophilia A (Factor VIII) deficiency. Students can use this Web site to help with their conclusion research.

Supplemental Document
Title: Genetic Counselor Report Rubric
Description: This file is to be used to evaluate the genetic counselor report. Each student will write a final report that includes all of the information that they have learned during this lesson.

Assessment

The students will be evaluated on the activity questions using the "Hemophilia Factor VIII Mutation Activity Answer Key" in Step 2, the "Student Directions: Physical Protein Model Building Answer Key" in Step 3, and "Factor VIII DNA Sequences Answer Key" in Steps 2 and 3. In addition, students will be evaluated on the group's written genetic counselor report using the "Counselor Report Rubric" provided in the Conclusion Step.

Modifications

Extensions
Students could research other proteins in which mutations cause disease (i.e. Hemophilia B and factor IX). Files of related proteins could be located using the Protein Data Bank Web site included below in the Web resources section. In addition, RASMOL included in the Web resources for Step 3 could be used to make observations about the protein's structure.

Web Resource
Title: Protein Data Bank
URL: http://www.rcsb.org/pdb/
Description: The Protein Data bank can be used to find information and PDB files of any protein structure that has been determined. The PDB files then can be downloaded and viewed and manipulated using molecular visualization software.

Other Modifications

For lower levels (either lower grades or general biology students) this activity could be modified by making the DNA sequence shorter or having less family members. Also, the use of RASMOL could be omitted.

For higher levels adding the concept of introns and exons in genes could modify this activity. More in depth coverage of protein folding principles could be added to the modeling portion.

Education Standards

National Science Education Standards
LIFE SCIENCE, CONTENT STANDARD C:
As a result of their activities in grades 9-12, all students should develop understanding of

- The cell
- **Molecular basis of heredity**
- Biological evolution
- Interdependence of organisms
- Matter, energy, and organization in living systems
- Behavior of organisms

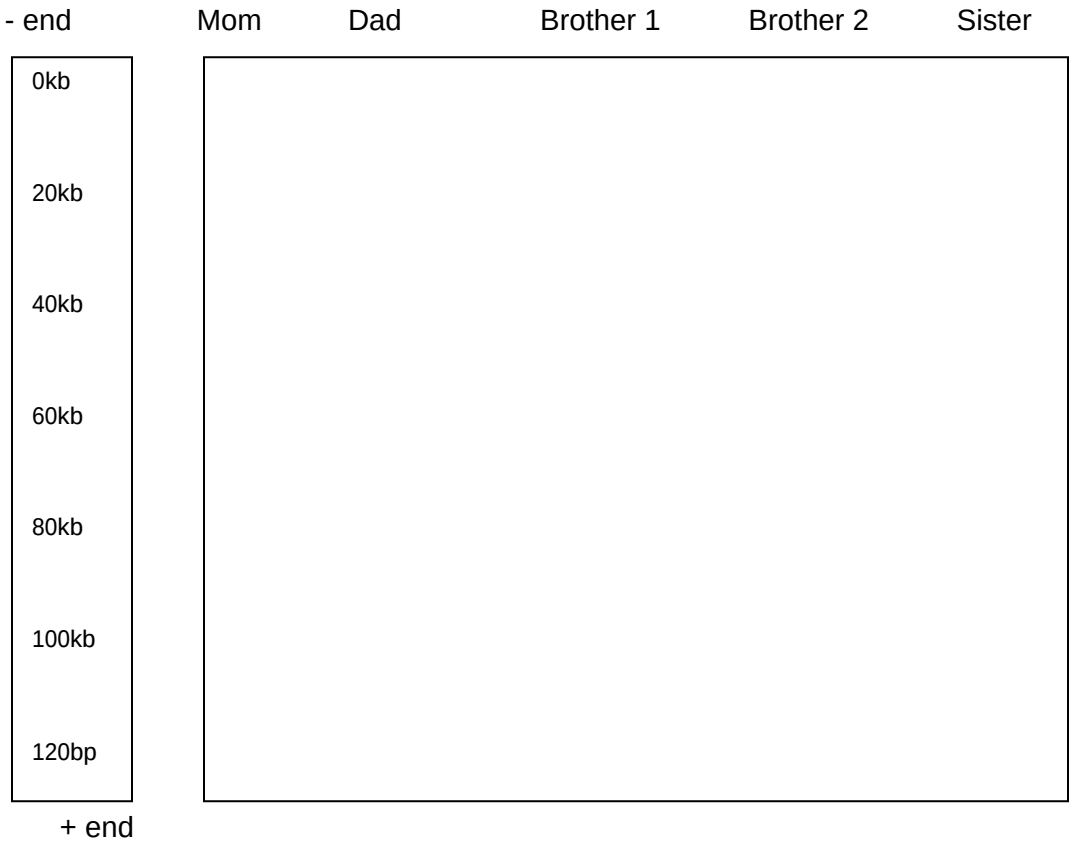
SCIENCE AND TECHNOLOGY, CONTENT STANDARD E:
As a result of activities in grades 9-12, all students should develop

- Abilities of technological design
- **Understandings about science and technology**

Electrophoresis Gel Paper Template

The Clark Family Story: Tracing the Cause of Hemophilia
Trish Strohfeldt, CDC's 2005 Science Ambassador Program

Directions
This is a template for the teacher to use to make a full sized one on large white paper for each group to use when they complete the simulated gel electrophoresis activity. Using a large piece of paper the teacher should copy the following template making one for each group.



Pretest Hemophilia Lesson Plan

The Clark Family Story: Tracing the Cause of Hemophilia
Trish Strohfeldt, CDC's 2005 Science Ambassador Program

1. **Explain what a sex-linked trait is. Give an example of a sex-linked trait or disorder.**

2. **Discuss how DNA results in the production of a protein in your body.**

3. **What is a pedigree and what information can you get from one?**

4. **How can changes in the DNA result in a change in the protein's shape?**

5. **What is the difference between hemophilia A and Hemophilia B?**

6. **What are some of the symptoms of hemophilia?**

7. **How can restriction enzymes and electrophoresis be used to determine if a person carries the hemophilia mutation or not?**

Pretest Hemophilia Lesson Plan Answer Key

The Clark Family Story: Tracing the Cause of Hemophilia
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1. **Explain what a sex-linked trait is. Give an example of a sex-linked trait or disorder.**
   A sex-linked trait is one that is controlled by a gene located on the X chromosome. Females have two copies of the X chromosome (and all the genes located on it) while males only have one copy. A mutation in one copy of the gene is usually not see in the phenotype of the female because of the actions of the remaining non-mutated gene. In males the phenotype is resulting from the only copy of the gene that they have. The Y chromosome in males does not carry another copy of the gene. (1)

   Hemophilia, color blindness (1)

2. **Discuss how DNA results in the production of a protein in your body.**
   DNA is transcribed into mRNA in the nucleus and the mRNA is translated into amino acids in at a ribosome. The amino acids are joined to form a protein by peptide bonds. (1)

3. **What is a pedigree and what information can you get from one?**
   A pedigree is a chart of circle (for females) and squares (for males) that is able to show a family tree and trace the occurrence of a trait or disease. Family members on the pedigree with the trait are shaded in while people who only carry the trait are half shaded in. Pedigrees can be used to trace a trait and find out the likelihood of a trait being passed on or being expressed in a family. (1)

4. **How can changes in the DNA result in a change in the protein's shape?**
   When DNA experiences a mutation a nucleotide base may change. This change in base will be seen in the resulting mRNA that is made from the DNA. The mRNA may then translate into the wrong amino acid. One amino acid change in the resulting protein can have an affect on the way the protein folds and changes the shape of the protein. The changed protein will then not be able to carry out its function. (1)

5. **What is the difference between hemophilia A and Hemophilia B?**
   Hemophilia A involves a mutation in the factor VIII blood clotting factor gene and hemophilia B involves a mutation in the factor IX blood clotting factor gene. Both mutations do not let the process of blood clotting happen as it normally should. (2)

6. **What are some of the symptoms of hemophilia?**
   Symptoms include internal bleeding, blood in stool and urine, frequent nosebleeds, easy bruising, and bleeding into muscles and joints that leads to chronic arthritis. (2)

7. **How can restriction enzymes and electrophoresis be used to determine if a person carries the hemophilia mutation or not?**
   The mutation in the factor VIII gene results in a different DNA sequence than the normal factor VIII gene. A restriction enzyme cuts the DNA at a specific sequence. This produces different lengths of DNA that when run on a gel produce bands at different places. Each length of band moves at a different rate along the gel. The bands then can be compared to determine who has the mutation and who does not. (2)

References

1. Campbell, N. Reece, J.B., Mitchell, L.G., editors. Biology. 5th Edition. Redwood City (CA): The Benjamin/Cummings Publishing Company, Inc.; 1999.

2. National Hemophilia Foundation. Hemophilia A: Factor VIII Deficiency [online]. 2005. [cited 2005 Aug 3]. Available from URL: http://www.hemophilia.org/bdi/bdi_types1.htm.

Clark Family Background Information

The Clark Family History: Tracing the Cause of Hemophilia
Trish Strohfeldt, CDC's 2005 Science Ambassador Program

Directions
Read the following information to your students to begin the lesson. They should take any notes that they think would be important later as they try to pinpoint the cause of the disorder and how it is passed in the Clark family.

The Family
The Clark family consists of 5 members. Mrs. Clark is a 32 year old lady with a family history of hemophilia A. She has a brother with hemophilia and her maternal grandfather also had hemophilia. Mr. Clark is 34 years old and has one brother and two sisters. He has no history of hemophilia in his family. The Clarks have three children and are considering having a fourth child. Their eldest child is a girl who is 8 years old. They also have two sons who are 2 years old and 5 years old.

The Disease
Hemophilia is a sex-linked disorder (gene is on the X chromosome). Since males have only one X chromosome and only carry one copy of the gene, if the gene is affected, they are usually affected with the disease. Females, who have two X chromosomes, will only be carriers if they have one affected copy of the gene. That is why they have the ability to pass the gene on to their offspring but are not affected with hemophilia themselves. The likelihood of a female having the disorder is possible but is very low since both her mother and her father would have to have and pass on the affected gene. (3)

There are two types of hemophilia, hemophilia A and hemophilia B. Hemophilia A involves a mutation in the factor VIII blood clotting factor gene and hemophilia B involves a mutation in the factor IX blood clotting factor gene. Both mutations do not let the process of blood clotting happen, as it normally should. Symptoms include internal bleeding, blood in stool and urine, frequent nosebleeds, easy bruising, and bleeding into muscles and joints that leads to chronic arthritis. Hemophilia affects 1 in every 10,000 males with 80% having type A and 20% having type B. Worldwide there are 500,000 people with hemophilia and there are 17,000 people with hemophilia in the US. (2)

References
1. Online Mendelian Inheritance in Man. Hemophilia A [online]. 2005. [cited 2005 Aug 3]. Available from URL: http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=306700.

2. National Hemophilia Foundation. Hemophilia A: Factor VIII Deficiency [online]. 2005. [cited 2005 Aug 3]. Available from URL:http://www.hemophilia.org/bdi/bdi_types1.htm.

3. Campbell, N. Reece, J.B., Mitchell, L.G., editors. Biology. 5th Edition. Redwood City (CA): The Benjamin/Cummings Publishing Company, Inc.; 1999.

Student Template Factor VIII DNA Sequences

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Directions

Below are the DNA sequences from a part of the factor VIII blood clotting factor involved with a mutation that resulted in Hemophilia A for each of the members of the Kane family. Refer to your Hemophilia Family Factor VIII Mutation Activity Student Directions' for instructions on how to explore this mutation.

Mother
Factor VIII Gene
Allele 1

5'tgcctcaacc tcccaagtag ctgggactac aggcatgtgc

mRNA:

AA:

shape:

caccatgccc aacgaatttt tatatgttta tgagcgacgg

mRNA:

AA:

shape:

gatgtcgcca tgttggctag gctggtcttg aacgcccgac 3’

mRNA:

AA:

shape:

Mother
Factor VIII Gene
Allele 2

5’tgcctcaacc tcccaagtag ctgggactac aggcatgtgc

mRNA:

AA:

shape:

caccatgccc tatattttta ttagcgacgg gatttcgcca

mRNA:

AA:

shape:

tgttggctag aactaatttt gctggtcttg aactcccgac 3’

mRNA:

AA:

shape:

Father
Factor VIII Gene
Allele 1

5’tgcctcaacc tcccaagtag ctgggactac aggcatgtgc

mRNA:

AA:

shape:

caccatgccc aacgaatttt tatatgttta tgagcgacgg

mRNA:

AA:

shape:

gatgtcgcca tgttggctag gctggtcttg aacgcccgac 3’

mRNA:

AA:

shape:

Father
Factor VIII Gene
Allele 2
Y chromosome-no gene for VIII

Son(1)
Factor VIII Gene
Allele 1

5’ tgcctcaacc tcccaagtag ctgggactac aggcatgtgc

mRNA:

AA:

shape:

caccatgccc tatattttta ttagcgacgg gatttcgcca

mRNA:

AA:

shape:

tgttggctag aactaatttt gctggtcttg aactcccgac 3’

mRNA:

AA:

shape:

Son(1)
Factor VIII Gene
Allele 2
Y chromosome-no gene for VIII

Son (2)
Factor VIII Gene
Allele 1

5’tgcctcaacc tcccaagtag ctgggactac aggcatgtgc

mRNA:

AA:

shape:

caccatgccc aacgaatttt tatatgttta tgagcgacgg

mRNA:

AA:

shape:

gatgtcgcca tgttggctag gctggtcttg aacgcccgac 3’

mRNA:

AA:

shape:

Son (2)
Factor VIII Gene
Allele 2
Y chromosome-no gene for VIII

Daughter
Factor VIII Gene
Allele 1

5’tgcctcaacc tcccaagtag ctgggactac aggcatgtgc

mRNA:

AA:

shape:

caccatgccc aacgaatttt tatatgttta tgagcgacgg

mRNA:

AA:

shape:

gatgtcgcca tgttggctag gctggtcttg aacgcccgac 3’

mRNA:

AA:

shape:

Daughter
Factor VIII Gene
Allele 2

5’tgcctcaacc tcccaagtag ctgggactac aggcatgtgc

mRNA:

AA:

shape:

caccatgccc aacgaatttt tatatgttta tgagcgacgg

mRNA:

AA:

shape:

gatgtcgcca tgttggctag gctggtcttg aacgcccgac 3’

mRNA:

AA:

shape:

Name: _____

Hemophilia Factor VIII Mutation Activity Student Directions
Simulated Gel Electrophoresis

The Clark Family Story: Tracing the Cause of Hemophilia
Trish Strohfeldt, CDC's 2005 Science Ambassador Program

Background
Hemophilia A and B are both caused by mutations in blood clotting factor genes. A mutation in the DNA strand of a blood clotting factor gene results in a protein that is not able to function properly and not able to catalyze a step in the blood clotting process. In this activity you will be looking at a short segment of the DNA nucleotide sequence from the family members and simulated restriction enzymes analysis to determine who has the mutation and where the mutation occurs in the DNA.

Directions

1. Obtain one "Student Template Factor VIII DNA Sequence" sheet for your group.
2. Assign each group member one or two family members' DNA to cut out and tape together. Make sure to include the empty spaces below the DNA labeled mRNA, AA and Shape. These spaces will be used in a later step. Make sure to label each person's DNA and tape the strand together in the correct order.

   Please answer the following questions:

   a. Why do the mother and daughter have two alleles for the factor VIII gene and the father and sons only have one allele?

   b. What is the difference between the 5' and 3' ends of the DNA strands?

   c. Remember, each DNA molecule has 2 strands. Each somatic cell in your body has two copies of each chromosome and therefore two copies of each allele for each gene (except the sex chromosomes in males). In this activity you only are looking at one DNA strand of each allele.

3. Use the following restriction enzyme (Tsp509I) to cut your DNA fragments. You will need to research where the cut site is for this particular restriction enzyme.

   a. Where does Tsp509I cut: _____

      Source of information (textbook, website?): _____

4. Using the paper electrophoresis gel, insert the DNA from each person into the correct well. Count the number of base pairs in each DNA fragment and place then along the correct place on the gel.

Please answer the following questions:

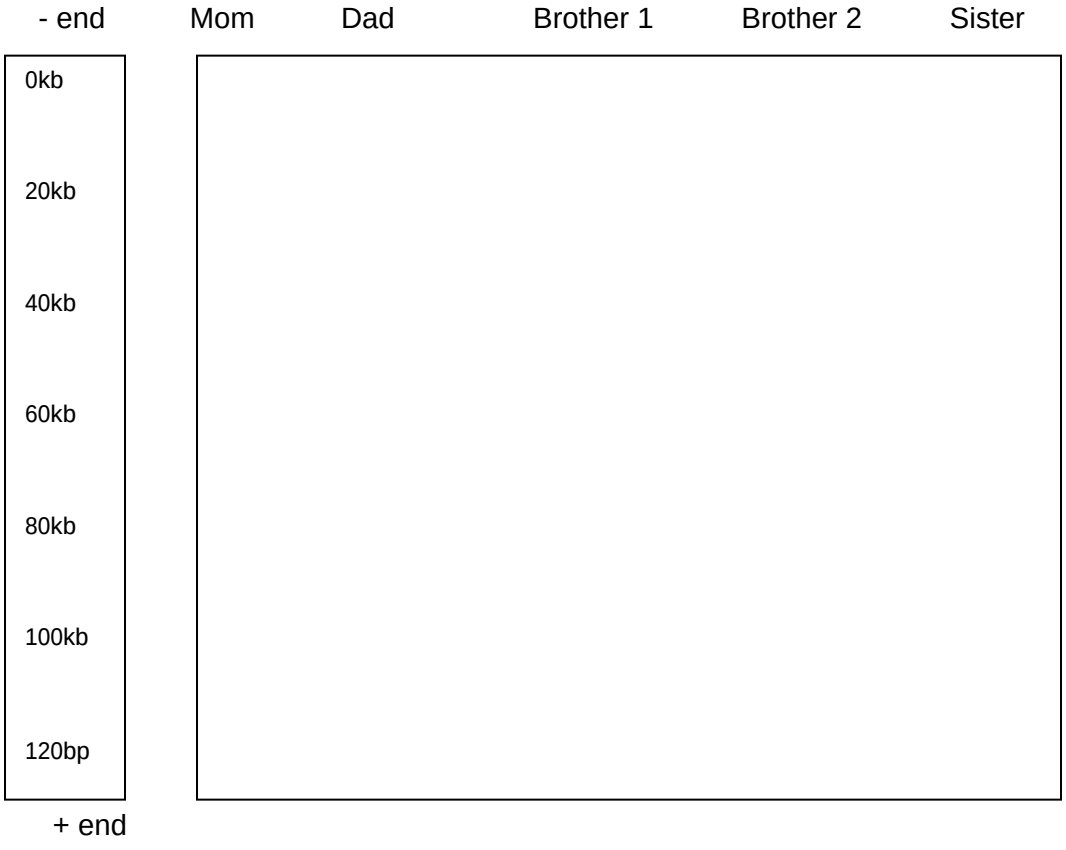
a. Which end of the gel is the DNA loaded? Explain why.

b. Explain what would happen if you loaded the DNA at the opposite end?

c. Why did you cut the enzymes with restriction enzymes before running them on the gel?

d. What would happen if you ran the DNA on the gel without cutting the DNA with restriction enzymes first?

e. In the box below, draw the results of your DNA electrophoresis.



f. Explain the results of the DNA test for the Hemophilia A mutation in factor VIII for each of the family members.

Name: _____

Hemophilia Factor VIII Mutation Activity Answer Key
Simulated Gel Electrophoresis

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Background
Hemophilia A and B are both caused by mutations in blood clotting factor genes. A mutation in the DNA strand of a blood clotting factor gene results in a protein that is not able to function properly and not able to catalyze a step in the blood clotting process. In this activity you will be looking at a short segment of the DNA nucleotide sequence from the family members and simulated restriction enzymes analysis to determine who has the mutation and where the mutation occurs in the DNA.

Directions

5. Obtain one "Student Template Factor VIII DNA Sequence" sheet for your group.
6. Assign each group member one or two family members' DNA to cut out and tape together. Make sure to include the empty spaces below the DNA labeled mRNA, AA and Shape. These spaces will be used in a later step. Make sure to label each person's DNA and tape the strand together in the correct order.

   Please answer the following questions:

   a. Why do the mother and daughter have two alleles for the factor VIII gene and the father and sons only have one allele?
   The gene for hemophilia is carried only on the X chromosome. Males have only one X while females have two. (1,2)

   b. What is the difference between the 5' and 3' ends of the DNA strands?
   The 3' and 5' refers to the carbon on the deoxyribose sugar that is located at the ends of the DNA strand. It is a way of designating direction on a DNA strand. (1)

   c. Remember, each DNA molecule has 2 strands. Each somatic cell in your body has two copies of each chromosome and therefore two copies of each allele for each gene (except the sex chromosomes in males). In this activity you only are looking at one DNA strand of each allele.

7. Use the following restriction enzyme (Tsp509I) to cut your DNA fragments. You will need to research where the cut site is for this particular restriction enzyme.

   a. Where does Tsp509I cut: **5' /A A T T 3'**
   3' T T A A/ 5'

   (3)

8. Using the paper electrophoresis gel, insert the DNA from each person into the correct well. Count the number of base pairs in each DNA fragment and place then along the correct place on the gel.

   Please answer the following questions:

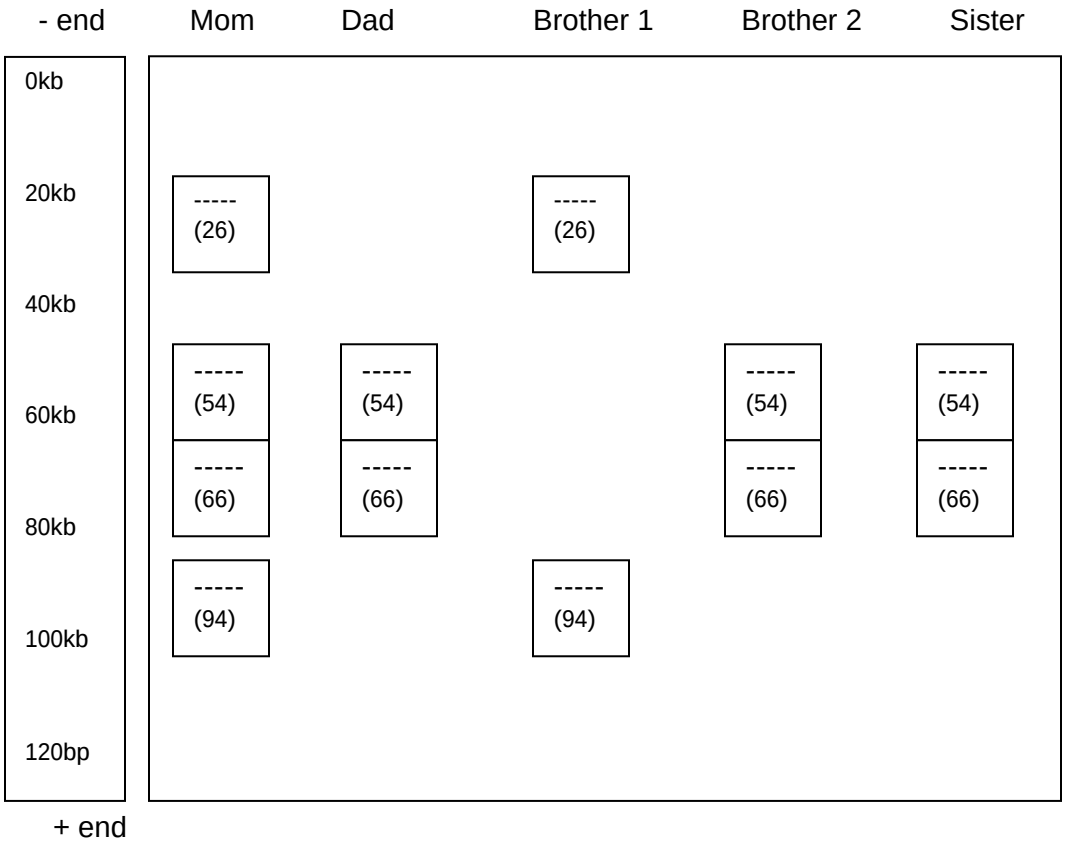
   f. Which end of the gel is the DNA loaded? Explain why.
   The DNA is loaded at the negative end of the gel. This is because DNA has a negative charge. When the current is run through the gel the negative DNA (due to the phosphate backbone) will move toward the positive end of the gel. (1)

   g. Explain what would happen if you loaded the DNA at the opposite end?
   The DNA would move toward the positive end, running off the gel before the fragments had a chance to separate. (1)

   h. Why did you cut the enzymes with restriction enzymes before running them on the gel?
   Electrophoresis separates DNA fragments based on their size (number of base pairs long). All the fragments of DNA are the same size until you use a restriction enzyme to cut the DNA in to smaller fragments. The enzyme cuts in a specific spot creating different sizes of DNA depending on the sequence of bases. The different lengths of DNA can then be seen by the distance they migrate through the gel. The longer fragments will move slower and the shorter fragment will move faster. (1)

   i. What would happen if you ran the DNA on the gel without cutting the DNA with restriction enzymes first?
   All the DNA strands would be the same length and appear in the same spot on the gel. You would be unable to tell who carries the mutation. (1)

In the box below, draw the results of your DNA electrophoresis.



f. Explain the results of the DNA test for the Hemophilia A mutation in factor VIII for each of the family members.

Mom

Mom has four bands (at 26bp, 54bp, 66bp, and 94bp) Females carry two copies of the factor VIII gene. She has a mutation in one copy of the gene producing the 26bp and 94bp bands. This shows that the mutation in the gene changes the DNA sequence so when the restriction enzyme cuts the strand it cuts in a different spot (creating two different length bands) in the muted and non-mutated versions.

Dad

Dad only carries one copy of the gene (since he only has once X chromosome) and it is a normal version (bands at 54bp and 66bp).

Brother 1

Brother 1 carries one copy of the gene and it is a mutated version producing bands at 29bp and 94bp.

Brother 2

Brother 2 caries one copy of the gene and it is a normal version with bands at 54bp and 66bp.

Sister

Sister carries two copies of the gene and both are normal. There are two bands at 54bp and two bands at 66bp.

References

1. Campbell, N. Reece, J.B., Mitchell, L.G., editors. Biology. 5th Edition. Redwood City (CA): The Benjamin/Cummings Publishing Company, Inc.; 1999.

2. National Hemophilia Foundation. Hemophilia A: Factor VIII Deficiency [online]. 2005. [cited 2005 Aug 3]. Available from URL:http://www.hemophilia.org/bdi/bdi_types1.htm.

3. New England Biolabs, Inc. NEBcutter V2.0. [21 July 2005]. Available from URL: http://tools.neb.com/NEBcutter2/index.php.

Name: _____

Physical Protein Model Building:
Making a Physical Model of the Factor VIII Clotting Cascade

The Clark Family Story: Tracing the Cause of Hemophilia
Trish Strohfeldt
CDC's 2005 Science Ambassador Program

Background

In this activity the student will create a physical model of the proteins coded for by the DNA sequences of each of the Clark family members. The sequence of nucleotide bases in DNA is transcribed into mRNA in the nucleus of the cell. The mRNA is then processed (this is when introns are spliced out) and the mRNA travels to the ribosome where the sequence is translated into a sequence of amino acids (primary structure). The sequence of amino acids and their properties determines the secondary and tertiary structure of the protein. The main two secondary structures of a protein are alpha helices and beta sheets. Differences in DNA sequences (mutations) can cause a change in the sequence of amino acids resulting in a protein with a different structure. Often, a change in the shape of a protein can be dramatic enough that its function is impaired or stopped.

Materials

- Biology textbook for reference (chapters on DNA structure and protein synthesis) or Web Resource: Dolan DNA Learning Center. DNA: Code. 2003. [22 July 2005]. Available from URL: http://www.dnai.org/a/index.html.
- Floral wire
- Genetic Code Table (in textbook)
- Ruler
- Permanent marker
- Pencil

Directions

Each student will create a physical model of one of the family member's alleles. Use your textbook to help you answer the questions as you work through the activity. Record the family member and allele below:

1. Family Member: _____ Allele: _____

2. Looking at the DNA sequence for your assigned family member and allele, use a pencil to transcribe the DNA into mRNA.

   Where does this process actually happen in the cell?

   When does this process actually occur in your cell (why?)

3. Using the Genetic Code Table, translate the mRNA sequence into the correct amino acids. Write down the amino acids under the mRNA sequence.

Where does this process actually happen in the cell?

When does this process actually occur in your cell (why?)

4. Using the amino acid and protein shape chart at the end of this worksheet, write down the secondary shape that the amino will take on (alpha helix-H, bend-B, and beta sheet-S) below the amino acid.

   What about amino acids make them take on different shapes in protein?

5. Obtain one 40cm piece of floral wire. Using a permanent marker, mark off 1cm segments on the wire. Each segment will correspond to one amino acid in the protein.

   What are some weaknesses of this model (think about proteins, sizes, environments, etc.)?

6. Using the amino acid shape sequence and the wire bend each 1cm segment into the correct shape in the correct order.

   To make:
   a. -helix: wind the segments around a pencil (like a phone cord)
   b. -beta sheet: bend each segment into a zigzag (one segment is the other the zag)
   c. -bend: bend the wire back on itself so that the net segment runs in the opposite direction

7. Compare the shape of a normal Factor VIII protein to a Factor VIII with a mutation. (which applies to this family and their sequence changes).

   Are there any silent mutations in the DNA? Explain.

   Are there any missense mutations in the DNA? Explain.

   Are there any nonsense mutations in the DNA? Explain.

   How do the shapes of the non-mutated and mutated versions of the protein differ?

Thinking about enzymes, explain why the change in Factor VIII shape might result in the disorder hemophilia.

Amino Acid and Shape Chart

Note: These secondary structures have been randomly assigned to each amino acid so that you can create your protein's structures easily. Most protein structures depend on many factors including pH, temperature, nearby amino acids, and more.

Amino Acid	Resulting Secondary Structure
cys	helix (forms disulfide bridges with other cys)
pro	bend
thr	beta sheet
asp	beta sheet
arg	beta sheet
ser	alpha helix
lys	beta sheet
tyr	beta sheet
ile	beta sheet
ala	beta sheet
met	alpha helix
gly	alpha helix
his	alpha helix
val	alpha helix
glu	alpha helix
asn	alpha helix
gln	alpha helix
leu	alpha helix

Name: _____

Physical Protein Model Building:
Making a Physical Model of the Factor VIII Clotting Cascade Protein

The Clark Family Story: Tracing the Cause of Hemophilia
Trish Strohfeldt
CDC's 2005 Science Ambassador Program

Background
In this activity the student will create a physical model of the proteins coded for by the DNA sequences of each of the Clark family members. The sequence of nucleotide bases in DNA is transcribed into mRNA in the nucleus of the cell. The mRNA is then processed (this is when introns are spliced out) and the mRNA travels to the ribosome where the sequence is translated into a sequence of amino acids (primary structure). The sequence of amino acids and their properties determines the secondary and tertiary structure of the protein. The main two secondary structures of a protein are alpha helices and beta sheets. Differences in DNA sequences (mutations) can cause a change in the sequence of amino acids resulting in a protein with a different structure. Often, a change in the shape of a protein can be dramatic enough that its function is impaired or stopped.

Materials
- Biology textbook for reference (chapters on DNA structure and protein synthesis) or Web Resource: Dolan DNA Learning Center. DNA: Code. 2003. [22 July 2005]. Available from URL: http://www.dnai.org/a/index.html.
- Floral wire
- Genetic Code Table (in textbook)
- Ruler
- Permanent marker
- Pencil

Directions
Each student will create a physical model of one of the family member's alleles. Use your textbook to help you answer the questions as you work through the activity. Record the family member and allele below:

4. Family Member: **varies** Allele: **1 or 2**

5. Looking at the DNA sequence for your assigned family member and allele, use a pencil to transcribe the DNA into mRNA.

   Where does this process actually happen in the cell?
   The process happens in the nucleus

   When does this process actually occur in your cell (why?)
   This process occurs when a cell needs to make a certain protein. A cell might need to make a protein to build new cells, make enzymes, etc.

6. Using the Genetic Code Table, translate the mRNA sequence into the correct amino acids. Write down the amino acids under the mRNA sequence.

Where does this process actually happen in the cell?
The process happens in the ribosome located in the cytoplasm of the cell.

When does this process actually occur in your cell (why?)
The process occurs when a cell needs to make a certain protein. A cell might need to build new cells, make enzymes, etc.

8. Using the amino acid and protein shape chart, write down the secondary shape that the amino will take on (alpha helix-H, bend-B, and beta sheet-S) below the amino acid.

   What about amino acids make them take on different shapes in protein?
   Each amino acid has different side chain groups with different chemical characteristics that determine how it interacts with the environment and with other amino acids. In addition, the environment the protein is in (solution, temperature, pH, etc.) affects a protein's shape.

9. Obtain one 40cm piece of floral wire. Using a permanent marker, mark off 1cm segments on the wire. Each segment will correspond to one amino acid in the protein.

   What are some weaknesses of this model (think about proteins, sizes, environments, etc.)?
   The model is not really as long as a true protein (not enough amino acids), all of the complex interactions can not be shown, the side chains are not visible, environmental factors (temperature, solution) need to be taken into account.

10. Using the amino acid shape sequence and the wire bend each 1cm segment into the correct shape in the correct order.

    To make:
    a. -helix: wind the segments around a pencil (like a phone cord)
    b. -beta sheet: bend each segment into a zigzag (one segment is the other the zag)
    c. -bend: bend the wire back on itself so that the net segment runs in the opposite direction

11. Compare the shape of a normal Factor VIII protein to a Factor VIII with a mutation (which applies to this family and their sequence changes).

    Are there any silent mutations in the DNA? Explain.
    No. All DNA base changes in the Factor VIII with a mutation result in a new amino acid. However, some amino acid changes do not change the shape of the protein.

    Are there any missense mutations in the DNA? Explain.
    Yes. A change in the DNA results in a change in the amino acid in codon numbers 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 3, 36, 37, 38

    Are there any nonsense mutations in the DNA? Explain.
    Yes. At codon number 38 a cys is changed to a stop codon.

How do the shapes of the non-mutated and mutated versions of the protein differ?
The first part of the protein is the same, but after codon number 17 the shape changes and the mutated protein is also two amino acids shorter.

Thinking about enzymes, explain why the change in Factor VIII shape might result in the disorder hemophilia.
When an enzyme's shape changes, it is no longer able to physically fit into the substrate that it usually fits. In the case of hemophilia, the factor VIII is an enzyme in the long cascade of reactions that results in blood clotting. If one enzyme is not able to do its job because of an incorrect shape it stops the reaction.

Amino Acid and Shape Chart

Note: These secondary structures have been randomly assigned to each amino acid so that you can create your protein's structures easily. Most protein structures depend on many factors including pH, temperature, nearby amino acids, and more.

Amino Acid	Resulting Secondary Structure
cys	helix (forms disulfide bridges with other cys)
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asp	beta sheet
arg	beta sheet
ser	alpha helix
lys	beta sheet
tyr	beta sheet
ile	beta sheet
ala	beta sheet
met	alpha helix
gly	alpha helix
his	alpha helix
val	alpha helix
glu	alpha helix
asn	alpha helix
gln	alpha helix
leu	alpha helix

Reference

Campbell, N. Reece, J.B., Mitchell, L.G., editors. Biology. 5th Edition. Redwood City (CA): The Benjamin/Cummings Publishing Company, Inc.; 1999.

Genetic Counselor Report Rubric

The Clark Family Story: Tracing the Cause of Hemophilia
Trish Strohfeldt, CDC's 2005 Science Ambassador Program

Directions
As the final assignment each student will create a genetic counselors report for the Clark family. The report should be written as if it were an actual genetic counselors report to the family members. The report should include the following:

Section 1: A Pedigree of the Clark Family

- Shows all family members mentioned
- Shows all affected individuals
- Shows all known carriers and suspected carriers

Section 2: Paragraph about the Clark Family's Hemophilia

- Explain who has the disorder and who does not
- Explain who in the family is a carrier and who could be a carrier
- Explain the risk of the next child having the disorder or being a carrier of the disorder (a Punnett square may be used to help explain this section)
- What gene the mutation is in
- What chromosome the mutation is located on
- What protein is affected by the mutation
- How the mutation causes a change in the protein's shape (may include digital photographs of the mutated and normal protein shape)

Section 3: Information about Hemophilia
In order to complete this section the student may need to do some additional research on the Internet or in their textbook. In addition to the sites included in the step, they may once again use the Web sites listed in part 2 and 3 of the lesson to gather information to be included in this section.

- Explain how Hemophilia is passed on and what a sex-linked trait is
- Explain the difference between hemophilia A and hemophilia B
- Discuss the signs and symptoms of hemophilia
- Discuss genetic testing for the disease
- Discuss the treatment options for hemophilia, including future treatments being researched